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(54) **PAUCILAMELLAR LIPID VESICLES USING CHARGE-LOCALIZED, SINGLE CHAIN, NONPHOSPHOLIPID SURFACTANTS.**

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CHEMICAL AND PHARMACEUTICAL BULLETIN vol. 35, no. 7, July 1987, TOKYO (JP)pages 2935 - 2942; H.KIWADA ET AL.: 'application of synthetic liposomes based on acyl amino acids or acyl peptides as drug carriers"

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Description

Reference to Related Applications

This application is a continuation-in-part of United States Patent Application Serial No. 157,571, filed March 3, 1988 now US Patent No.4 911 928, entitled "Paucilamellar Lipid Vesicles", which is a continuation-in-part of United States Patent Application No. 078,658, filed July 28, 1987, now United States Patent No. 4,855,090, which itself is a continuation-in-part of United States Patent Application Serial No, 025,525, filed March 13, 1987, now abandoned, both entitled "Method of Producing High Aqueous Volume Multilamellar Vesicles," and United States Patent Application Serial No. 124,824, filed November 25, 1987 now US Patent No.4 917 951, entitled "Lipid Vesicles Formed of Surfactants and Steroids." This application is also related to United States Patent Application Serial No. 163,806 now US Patent No.4 895 452 entitled "Method and Apparatus for Producing Lipid Vesicles".

Background of the invention

The present invention relates to the production of paucilamellar lipid vesicles having charge-localized, single chain nonphospholipid zwitterionic or anionic surfactants as the primary structural material of their lipid bilayers. More particularly, the present invention relates to a method of producing these paucilamellar lipid vesicles having a large aqueous or organic liquid filled amorphous central cavity, as well as the vesicles themselves.

Lipid vesicles are substantially spherical structures made of materials having a high lipid content, e.g., surfactants or phospholipids. The lipids of these spherical vesicles are organized in the form of lipid bilayers. The lipid bilayers encapsulate an aqueous volume which is either interspersed between multiple onion-like shells of lipid bilayers, forming a classic multilamellar lipid vesicle ("MLV"), or the aqueous volume may be contained within an amorphous central cavity. Common lipid vesicles having an amorphous central cavity filled with aqueous medium are the unilamellar lipid vesicles. Large unilamellar vesicles ("LUV"'s) generally have a diameter greater than about 1μm while small unilamellar lipid vesicles ("SUV"'s) generally have a diameter of less than 0.2μm.

Paucilamellar lipid vesicles ("PLV"'s) are a hybrid having features of both MLV's and LUV's. PLV's are characterized by having 2-10 peripheral bilayers surrounding a large, unstructured central cavity.

The potential utility of liposomes is widely recognized. Their ability to encapsulate aqueous volumes and/or lipophilic material makes them attractive devices for transporting a whole spectrum of molecules, including macromolecules and drugs, vaccines, and other therapeutic compositions. In addition, it is possible to encapsulate supramolecular structures such as viruses using classes of liposomes. Some types of vesicles have shown an ability to act as adjuvants or as carriers or storage devices for oil-based materials.

Each type of lipid vesicle appears to have certain uses for which it is best adapted. For example, the multiple onion-like lipid bilayers of classic MLV's provide this lipid vesicle with increased durability and protection from enzymatic degradation. The multiple shells greatly diminish the volume available for aqueous solutions to be encapsulated within the bilayers of the MLV. MLV's have heretofore been deemed most advantageous for carrying lipophilic materials which can be incorporated in their bilayers. However, there is a maximum amount of lipophilic material that can be incorporated into MLV bilayers, beyond which the bilayers become unstable and these vesicles break down. In contrast, the single shell of LUV's allow the encapsulation of a larger volume of aqueous material but because of their single lipid bilayer structure, LUV's are not as physically durable as MLV's. SUV's have neither the lipid or aqueous volumes of MLV's or LUV's, but because of their small size have easiest access to cells and tissues.

PLV's appear to have advantages as transport vehicles for many uses as compared with the other types of lipid vesicles. In particular, because of their large unstructured central cavity, PLV's are easily adapted for transport of large quantities of aqueous-based materials. However, their multiple lipid bilayers provide PLV's with the ability to carry lipophilic material in their bilayers as well as with additional physical strength and resistance to degradation as compared with the single lipid bilayer of the LUV. In addition, as illustrated in the present application and United States Patent Application Serial No. 157,571, now US Patent No. 4 911 928, the central cavity of the PLV's can be filled wholly or in part with an apolar oil or wax and then can be used as a vehicle for the transport or storage of hydrophobic materials. Thus, the amount of hydrophobic material which can be transported by PLV's with an apolar core is much greater than can be transported by classic MLV'S.

Early lipid vesicle or liposome studies used phospholipids as the lipid source for bilayers, primarily because phospholipids are the principal structural components of natural membranes. However, there are a number of problems associated with using phospholipids as artificial membranes. First, isolated phospholipids are subject to degradation by a large variety of enzymes. Second, the most easily available phospholipids are those from natural sources, e.g., egg yolk lecithin, which contain polyunsaturated acyl chains that are subject to autocatalyzed peroxidation. When peroxidation occurs, the lipid structure breaks down, causing premature release of encapsulated materials and the formation of toxic peroxidation byproducts. This problem can be avoided by hydrogenation but hydrogenation is an expensive process, thereby raising the cost of the starting materials. Cost is a third problem associated with the use of phospholipids on a large scale. The high cost of a kilogram of egg yolk lecithin pure enough for pharmacological liposome production places a severe limitation on the use of phospholipids as a source material.

It is now known that commercially available surfactants may be used to form the lipid bilayer in a variety of lipid vesicles. (See, e.g., U.S. Patent Serial No. 4,217,344, U.S. Patent Serial No. 4,855,090, and U.S. Patent Application Serial No. 157,571 now US Patent No. 4 911 928). WO-A-8 806 883 claims priority from US Patent Application Serial No. 157,571. Both surfactants and phospholipids are amphiphiles, having at least one lipophilic acyl or alkyl group attached to a hydrophilic head group. The head groups are attached to one or more lipophilic chains by ester, ether or amide linkages. Commercially available surfactants include the BRIJ family of polyoxyethylene fatty acid ethers, the SPAN sorbitan alkyl esters, and the TWEEN polyoxyethylene sorbitan fatty acid esters, all available from ICI Americas, Inc., of Wilmington, Delaware. Unlike phospholipids, these surfactants are generally nonionic, and addition of a charge-producing amphiphile is usually required to prevent floculation and to increase the degree of encapsulation of water-soluble substances. Addition of a charge-producing amphiphile is not required if the primary wall lipid is anionic -- as in the case of sarcosinamides.

In addition, the presence of a sterol or sterol-like molecule in the lipophilic phase used to create the lipid bilayer has often been found to be important for increasing the stability of the bilayer and hence the vesicle.

In 1982, Murakami et al. in J. Org. Chem., 43, 2137-2144 disclosed the preparation of small (.05-.2 μm), single- or multiple-walled vesicles capable of encapsulating aqueous volumes, using cationic and zwitterionic double chain amphiphiles synthesized to mimic the structure of naturally occurring phospholipids. The aqueous-carrying capacity of these vesicles is unknown as is their structure and stability. Murakami does not mention the possibility of oil encapsulation or single chain varieties of these ionic lipids.

The use of anionic or zwitterionic surfactants as cleaning or conditioning agents in cleansers such as shampoos is well documented in the art (see e.g., U.S. Patent Serial No. 4,075,131 and U.S. Patent Serial No. 4,832,872). However, in their present formulation in cleanser compositions, these surfactants are not present in vesicle form.

Recently an improved method for creating large aqueous volume MLV'S and PLV's using. commercially available, synthetic, nonionic surfactants has been discovered. U.S. Patent No. 4,855,090, and U.S. Patent Application Serial No. 157,571 now US Patent No. 4 911 928 disclose this new method which has the advantage of being faster and more cost-efficient than previous methods. This improved method of creating PLV's and large aqueous volume MLV's, which is applicable to only certain surfactants, forms vesicles in less than a second rather than the minutes or hours of classical techniques. Moreover, the improved method allows vesicles to be formed without the use of solvents and without the formation of a separable lamellar phase. These techniques, and the devices to utilize them, have only been described in the aforementioned patents, as well as the related applications. In contrast, the classic methods for producing multilamellar lipid vesicles are well-documented in the art. See for example Gregoriadis, G., ed. Liposome Technology (CRC, Boca Raton, FL), Vols. 1-3 (1984), and Dousset and Douste-Blzay (in Les Liposomes, Puisieux and Delattre, ed., Techniques et Documentation Lavoiser, Paris, pp. 41-73 (1985).

No matter how the MLV's or PLV's are formed, once made it is necessary to determine the effectiveness of the process. Two measurements commonly used to determine the effectiveness of encapsulation of materials in lipid vesicles are the encapsulated mass and captured volume. The encapsulated mass is the mass of the substance encapsulated per unit mass of the lipid and is often given as a percentage. The captured volume is defined as the amount of the aqueous phase trapped inside the vesicle divided by the amount of lipid in the vesicle structure, normally given in ml liquid/g lipid.

The methods and materials disclosed herein for producing paucilamellar lipid vesicles formed of single chain charge-localized nonphospholipid zwitterionic or anionic surfactants all yield stable vesicles capable of encapsulating aqueous or oil volumes.

Accordingly, an object of the invention is to provide stable paucilamellar lipid vesicles from charge-localized non-phospholipid single chain surfactants.

Another object of the invention is to provide a method for producing such paucilamellar lipid vesicles which is rapid and uses relatively inexpensive materials.

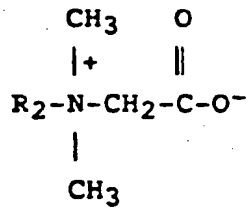
A further object of the invention is to provide a vehicle for the transport of aqueous or oil-soluble materials formed essentially of charge-localized nonphospholipid single chain zwitterionic or anionic surfactants.

These and other objects and features of the invention will be apparent from the detailed description and the claims.

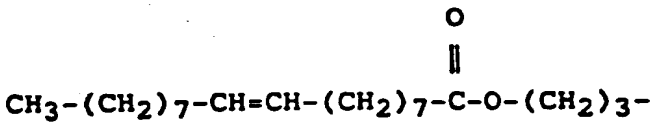
Summary of the Invention

The present invention features paucilamellar lipid vesicles whose primary lipid bilayer structural material is charge-localized single chain nonphospholipid material such as betaines or anionic sarcosinamides, for use as carriers of hydrophilic or hydrophobic materials, and a method for their manufacture. A "charge-localized" molecule, as defined herein is a molecule containing a separation of charge so that a positive charge is located at one portion of the molecule while a negative charge is located at a different portion -- for example, a zwitterionic or an ionic molecule which has an associated counter-ion.

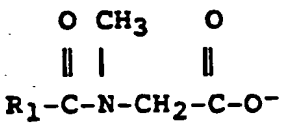
The method of the present invention for making paucilamellar lipid vesicles has the steps of forming a lipophilic phase of a single chain nonphospholipid zwitterionic or anionic surfactant and any other lipid soluble materials being incorporated in the bilayers of the vesicle which are dissoluble in the surfactant. Zwitterionic paucilamellar lipid vesicles are preferably made from surfactants selected from the group consisting of betaines having the structure



where R_2 is a long chain fatty acid ester. A preferred betaine is oleoyl propyl betaine, where R_2 has the structure



Anionic surfactants preferred in the invention are selected from the group consisting of sarcosinamides having the formula



where

is the carbonyl derivative of a long chain fatty acid having 12 to 20 carbon atoms. Preferred sarcosinamides include the sarcosinamides of lauric acid, oleic acid, or methyl-sarcosinamides of mixed fatty acids having 14-20 carbon atoms, e.g., the methyl-sarcosinamides of fraction 3 of coconut oil.

The lipophilic phase, which may contain charge-producing materials and/or sterols such as cholesterol or hydrocortisone or their analogs and derivatives, is blended with an aqueous phase consisting of an aqueous buffer and any aqueous-soluble materials to be encapsulated, under shear mixing conditions, to form the paucilamellar lipid vesicles. "Shear mixing" is defined as the mixing of the lipophilic phase with the aqueous phase under turbulent or shear conditions which provide adequate mixing to hydrate the lipid and form lipid vesicles. "Shear mixing" is achieved by liquid shear which is substantially equivalent to a relative flow rate for the combined phases of about 5-30 m/s through a 1 mm orifice.

The invention further features the encapsulation of oil-soluble or oil-suspendable materials within these paucilamellar lipid vesicles. This procedure commences with dispersing the material to be encapsulated in an oil or wax, forming an oily phase. The oil or wax is a water immiscible oily solution selected from a group consisting of oils, waxes, natural and synthetic triglycerides, acyl esters, and petroleum derivatives, and their analogs and derivatives. The terms "disperse" or "dispersion" as used herein include dissolution or forming a suspension or colloid to yield a flowable phase. The oily phase containing the oil-dispersible material is mixed with the lipid phase and the combined oil-lipid phase is blended under shear mixing conditions with the aqueous phase. Surfactants useful in the encapsulation process are the same as those used to make the aqueous-filled paucilamellar lipid vesicles described above.

In order to achieve the proper blending necessary to form paucilamellar lipid vesicles of this invention, all of the materials are normally in a flowable state. This is easily achieved by elevating the temperature of the lipophilic phase in order to make it flowable followed by carrying out the shear mixing between the lipophilic phase and the aqueous phase at a temperature such that both phases are liquids. The surfactants of this invention are such that only gentle heating is required to obtain flowability. While it is often desirable to use the same temperature for both phases, this is not always necessary.

Detailed Description of Preferred Embodiments

The present invention relates to the production of zwitterionic and anionic paucilamellar lipid vesicles and the zwitterionic or anionic paucilamellar lipid vesicles themselves. These lipid vesicles, which have a single chain, nonphospholipid charge-localized surfactant material as their primary structural component, are characterized by having 2-10 lipid bilayers with a small aqueous volume separating each substantially spherical lipid shell, surrounding a large amorphous central cavity. The cavity can be filled with an oil (including a wax), an aqueous-based solution or some mixture thereof.

For certain uses, the incorporation of a charge-producing amphiphile or a sterol may be desired. Preferred charge-producing amphiphiles include dicetyl phosphate, cetyl sulfate, long chain fatty acids, retinoic acid, carboxylic acids, quarternary ammonium compounds, and derivates thereof. Cholesterol or one of its derivatives is a preferred sterol.

The paucilamellar lipid vesicles can be made by a variety of devices which provides sufficiently high shear for shear mixing. There are a large variety of these devices available on the market including a microfluidizer such as is made by Biotechnology Development Corporation, a "French"-type press, or some other device which provides a high enough shear force and the ability to handle heated, semiviscous lipids. If a very high shear device is used, it may be possible to microemulsify powdered lipids, under pressure, at a temperature below their normal melting points and still form the lipid vesicles of the present invention.

A device which is particularly useful for making the lipid vesicles of the present invention has been developed by Micro Vesicular Systems, Inc., Vineland, New Jersey and is further described in United States Patent Application Serial No. 163,806 Now US Patent No. 4 895 452. Briefly, this device has a Substantially cylindrical mixing chamber with at least one tangentially located inlet orifice. One or more orifices lead to a reservoir of the lipophilic phase, mixed with an oil phase if lipid-core PLV's are to be formed, and at least one of the other orifices is attached to a reservoir for the aqueous phase. The different phases are driven

into the cylindrical chamber through pumps, e.g., positive displacement pumps, and intersect in such a manner as to form a turbulent flow within the chamber. The paucilamellar lipid vesicles form rapidly, e.g., in less than 1 second, and are removed from the chamber through an axially located discharge orifice. In a preferred embodiment, there are four tangentially located inlet orifices and the lipid and aqueous phases are drawn from reservoirs, through positive displacement pumps, to alternating orifices. The fluid stream through the tangential orifices is guided in a spiral flow path from each inlet or injection orifice to the discharge orifice. The flow paths are controlled by the orientation or placement of the inlet or injection orifices so as to create a mixing zone by the intersection of the streams of liquid. The pump speeds, as well as the orifice and feed line diameters, are selected to achieve proper shear mixing for lipid vesicle formation. As noted, in most circumstances, turbulent flow is selected to provide adequate mixing.

The invention, and its many uses, will be more apparent from the following, non-limiting examples.

Example 1:

In this example three different sarcosinamides are tested for their ability to form paucilamellar lipid vesicles in the presence and absence of cholesterol and oleic acid, and for their ability to encapsulate an aqueous solution.

Table 1 lists the materials used and the results. The presence or absence of cholesterol (C) is indicated by a positive (+) or negative (-) sign. All sarcosinamides are obtained from R.T. Vanderbilt Company, Inc. (Norwalk, CT): Vanseal LS ("LS") is the sarcosinamide of lauric acid, Vanseal OS ("OS") is the sarcosinamide of oleic acid, and Vanseal CS ("CS") is a methyl-sarcosinamide of fatty acids derived from coconut oil - a mixture of mostly saturated C_{14}-C_{20} carboxylic acids. The reactions are carried out in solutions having a pH such that less than 60% of the carboxyl groups are dissociated (pH range 3-5.5). Although syringes are used to provide the shear mixing in this and the following examples, any shear-producing device which provides shear mixing can be used.

One ml of the lipophilic phase formed of the surfactant (and additives, when present) is placed in a 10 ml syringe and heated to 45°C, a temperature above the melting point of the surfactant. The lipophilic phase which results after the heating and blending of the lipophilic component(s) is forcibly injected, via a three-way stop-cock, into 4 ml of an aqueous phase. The aqueous phase (in this example, 4 mls of water) is contained in a 10 ml syringe, and is also at 45°C. The process of injection of the lipophilic phase into the aqueous phase takes less than five seconds. The resulting mixture is then forced repeatedly between the syringes at a linear flow rate of 8-12 m/s through an orifice about 1 mm in diameter. The mixture is driven continuously back and forth between the two syringes for approximately 2 minutes, providing the shear mixing necessary to make the paucilamellar lipid vesicles. A milky suspension containing the paucilamellar lipid vesicles results. The lipid vesicles are separated by centrifugation at 10,000 rpm for 15 minutes in a Beckman Instrumental Co. J-21 centrifuge, forming a low density phase on top of the aqueous solution.

TABLE 1

Surfactant	C	H_2O Uptake ml/g	Diameter (μm)
LS	+	3.0	0.3
LS	-	4.0	0.45
OS	+	3.0	0.35
OS	-	4.0	0.60
CS	+	3.0	0.26
CS	-	2.5	0.40
Composition: surfactant/cholesterol = 33 mM/11 mM			

As is evident from the results listed in Table 1, all of these surfactants form water-encapsulating vesicles in the presence or absence of cholesterol. The diameters and encapsulated volumes are greater when vesicles are formed with surfactants alone.

Example 2:

In this example, mineral oil (Drakeol 19) is used to show oil encapsulation efficiency for the paucilamellar lipid vesicles of this invention. As in the previous example, the surfactants tested are sarcosinamides of

lauric acid, oleic acid and coconut oil fatty acids, and the lipophilic phase is formed with and without additives.

Table 2 lists the materials used, and the results. As in Table 1, the presence or absence of additives is indicated by +/-.

As in Example 1, the surfactant (and cholesterol, when present) is placed in a 10 ml syringe and heated to 45 °C, a temperature above the melting point of the surfactant, forming 1 ml of the lipophilic phase. This surfactant mixture is then blended with different amounts of mineral oil in a series of experiments until post-encapsulation oil saturation is reached. The lipophilic phase of the lipid and oil is then blended with 4 ml of water, using the syringe method of Example 1.

As is evident from Table 2, all of these surfactants are able to encapsulate oil in the presence or absence of cholesterol and oleic acid. As in Example 1, diameters and volumes encapsulated are greater when vesicles are formed with surfactants alone.

TABLE 2

Surfactant	C	Oil Uptake ml/g	Diameter (μm)
CS	+	10	0.68
CS	-	18	0.84
LS	+	7	0.34
LS	-	12	0.50
OS	+	7	0.16
OS	-	7	0.30
Composition: surfactant/cholesterol = 33 mM/11 mM			

Example 3:

In this example, the ability of oleoyl propyl betaine to encapsulate water or mineral oil (Drakeol 19) is measured. Materials and proportions used are listed in Table 3.

TABLE 3

Oleoyl propyl betaine Cholesterol	33 mM 15 mM
1 ml total lipid	

The example is performed following essentially the some protocol as those of Examples 1 and 2. The aqueous phase is 2 mls water.

TABLE 4

H_2O Uptake (ml/g)	Diameter (μm)
1.5	0.15
Oil Uptake (ml/g)	Diameter (μm)
15-16	-1.9

The results, listed in Table 4, clearly show the ability of oleoyl propyl betaine to encapsulate aqueous or oil volumes.

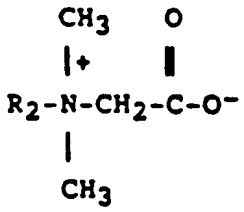
Claims

1. Paucilamellar lipid vesicles consisting of 2-10 lipid bilayers in the form of spherical shells separated by aqueous layers, said lipid bilayers comprising a charge-localized single chain nonphospholipid surfactant selected from the group consisting of betaines and anionic sarcosinamides as the primary lipid, and any lipid-soluble materials to be incorporated in said lipid vesicle bilayers.

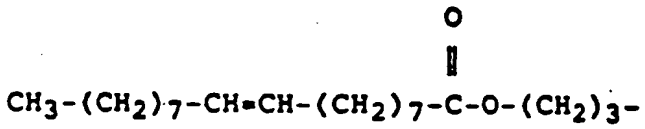
2. The paucilamellar lipid vesicles of claim 1 wherein the lipid bilayers surround an aqueous filled amorphous central cavity.

3. The paucilamellar lipid vesicle of claim 1 wherein the lipid bilayers surround an oil-filled amorphous central cavity.

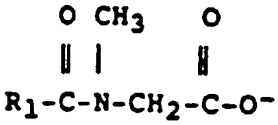
4. The paucilamellar lipid vesicles according to any one of claims 1 to 3 wherein said betaine comprises a betaine having the structure:



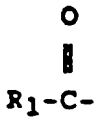
where R_2 is a long chain fatty acid ester, e.g. propyl oleate, having the structure:



5. The paucilamellar lipid vesicles according to any one of claims 1 to 3 wherein said anionic sarcosinamide comprises a sarcosinamide having the structure:



where



is the carbonyl group of a long chain fatty acid having 12 to 20 carbon atoms, and wherein for example said long chain fatty acid is selected from lauric acid, fatty acids having 14-20 carbon atoms, and mixtures thereof.

6. The paucilamellar lipid vesicles according to any one of claims 1 to 3 wherein said lipophilic phase further comprises a sterol, e.g. cholesterol.

7. The paucilamellar lipid vesicles according to any one of claims 1 to 3 wherein said lipophilic phase further comprises a charge-producing amphiphile.

8. A method of preparing the aqueous-filled, paucilamellar lipid vesicles of claim 2 consisting essentially of the steps of:

A. Providing a solventless nonaqueous lipophilic phase comprising a single chain charge-localized nonphospholipid surfactant selected from the group consisting of betaines and anionic sarcosinamides and any lipid-soluble materials to be incorporated in said lipid vesicle;

B. Providing an aqueous phase formed of an aqueous solvent and any aqueous soluble materials to be encapsulated; and

C. Combining said nonaqueous lipophilic phase with an excess of said aqueous phase in a single step under shear mixing conditions;

whereby said aqueous-filled paucilamellar lipid vesicles are formed without the formation of a separable lamellar phase.

9. A method of preparing the oil-filled paucilamellar lipid vesicles of claim 3 having a water-immiscible oily material in their amorphous central cavities consisting essentially of the steps of:

A. Providing a solventless nonaqueous lipid phase comprising a charge-localized single chain nonphospholipid surfactant selected from the group consisting of betaines and anionic sarcosinamides and any lipid-soluble materials to be incorporated in said lipid vesicle;

B. Providing an aqueous phase formed of an aqueous solvent and any aqueous soluble materials to be incorporated in said lipid vesicle; and

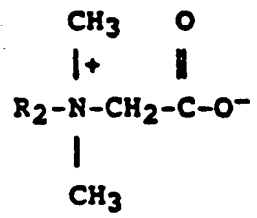
C. Providing an oil phase of a water-immiscible oily material and any material soluble therein to be incorporated in said lipid vesicle;

D. Forming a lipophilic phase by blending said lipid phase and said oil phase;

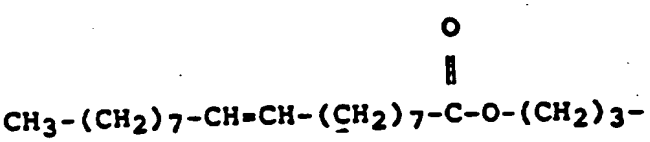
E. Combining said lipophilic phase with an excess of said aqueous phase in a single step under shear mixing conditions;

whereby said single chain charge-localized nonphospholipid paucilamellar lipid vesicles having a water-immiscible oil material in their central cavities are formed without the formation of a separable lamellar phase.

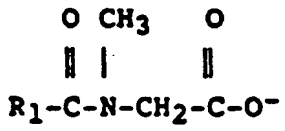
10. The method of claim 8 or claim 9 wherein said betaine comprises a betaine having the structure:



where R_2 is a long chain fatty acid ester, for example propyl oleate, having the structure:



11. The method of claim 8 or claim 9 wherein said sarcosinamide comprises a sarcosinamide having the structure



12. The method of claim 8 or claim 9 wherein said lipophilic phase further comprises a sterol, e.g. cholesterol.

13. The method of claim 8 or claim 9 wherein said lipophilic phase further comprises a charge-producing amphiphile.

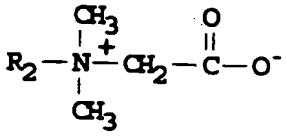
Patentansprüche

1. Paucilamellare Lipidvesikel bestehend aus 2 bis 10 Lipiddoppelschichten in der Form von kugelförmigen Hüllen, die durch wäßrige Schichten getrennt sind, wobei die Lipiddoppelschichten als primäres Lipid einen einkettigen, nichtphospholipiden, oberflächenaktiven Stoff mit lokalisierter Ladung aus der Gruppe der Betaine und anionischen Sarkosinamide enthalten oder daraus bestehen sowie beliebige, lipidlös-liche Stoffe, die in die Lipidvesikeldoppelschich-ten inkorporiert werden sollen.

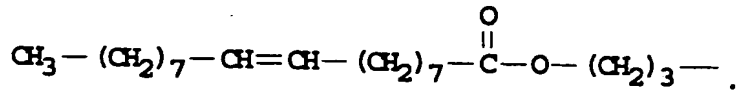
2. Paucilamellare Lipidvesikel gemäß Anspruch 1, worin die Lipiddoppelschichten eine wassergefüllte, amorphe Zentralhöhle umgeben.

3. Paucilamellare Lipidvesikel gemäß Anspruch 1, worin die Lipiddoppelschichten eine ölgefüllte, amorphe Zentralhöhle umgeben.

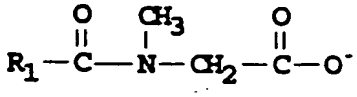
4. Paucilamellare Lipidvesikel nach einem der Ansprüche 1 bis 3, worin das Betain ein Betain folgender Struktur enthält oder daraus besteht:



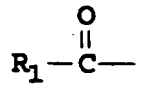
worin R_2 ein langkettiger Fettsäureester, Z. B. Propyloleat, mit folgender Struktur ist:



5. Paucilamellare Lipidvesikel nach einem der Ansprüche 1 bis 3, worin das anionische Sarkosinamid ein Sarkosinamid folgender Strukur enthält oder daraus besteht:



worin



die Carbonylgruppe einer langkettigen Fettsäure mit 12 bis 20 Kohlenstoffatomen ist und die langkettige Fettsäure z. B. Laurinsäure, Fettsäuren mit 14 bis 20 Kohlenstoffatomen oder deren Mischungen ist.

6. Paucilamellare Lipidvesikel nach einem der Ansprüche 1 bis 3, worin die lipophile Phase weiterhin Sterol, z. B. Cholesterol, enthält oder daraus besteht.

7. Paucilamellare Lipidvesikel nach einem der Ansprüche 1 bis 3, worin die lipophile Phase weiterhin eine ladungerzeugende, amphiphile Substanz enthält oder daraus besteht.

8. Verfahren zur Herstellung der wassergefüllten, paucilamellaren Lipidvesikel gemäß Anspruch 2, das im wesentlichen die Schritte umfaßt:

A. Bereitstellung einer lösemittelfreien, nichtwäßrigen, lipophilen Phase, die einen einkettigen, nichtphospholipiden, oberflächenaktiven Stoff mit lokalisierter Ladung aus der Gruppe der Betaine und anionischen Sarkosinamide enthält oder daraus besteht sowie beliebige, lipidlösliche Stoffe, die in das Lipidvesikel inkorporiert werden sollen;

B. Bereitstellung einer wäßrigen Phase gebildet aus einem wäßrigen Lösemittel und beliebigen, wasserlöslichen Stoffen, die eingekapselt werden sollen; und

C. Vereinigung der nichtwäßrigen, lipophilen Phase mit einem Überschuß der wäßrigen Phase in einem einzigen Schritt durch Mischen unter Scherbedingungen;

wodurch die wassergefüllten, paucilamellaren Lipidvesikel ohne die Ausbildung einer abtrennbaren, lamellaren Phase gebildet werden.

9. Verfahren zur Herstellung der ölgefüllten, paucilamellaren Lipidvesikel gemäß Anspruch 3, die in ihren amorphen Zentralhöhlen einen mit Wasser nicht mischbaren, öligen Stoff aufweisen, das im wesentlichen die Schritte umfaßt:

A. Bereitstellung einer lösemittelfreien, nichtwäßrigen, Lipidphase, die einen einkettigen, nichtphospholipiden, oberflächenaktiven Stoff mit lokalisierter Ladung aus der Gruppe der Betaine und anionischen Sarkosinamide enthält oder daraus besteht sowie beliebige, lipidlösliche Stoffe, die in das Lipidvesikel inkorporiert werden sollen;

B. Bereitstellung einer wäßrigen Phase gebildet aus einem wäßrigen Lösemittel und beliebigen, wasserlöslichen Stoffen, die in das Lipidvesikel inkorporiert werden sollen;

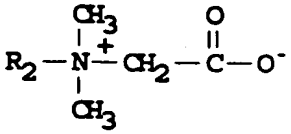
C. Bereitstellung einer Ölphase aus einem mit Wasser nicht mischbaren, öligen Stoff und einem beliebigen, darin löslichen Stoff, die in das Lipidvesikel inkorporiert werden sollen;

D. Bildung einer lipophilen Phase durch Mischen der Lipidphase und der Ölphase;

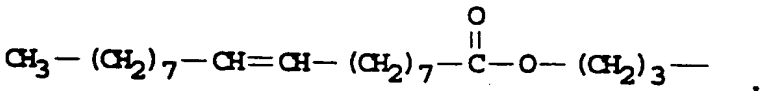
E. Vereinigung der lipophilen Phase mit einem Überschuß der wäßrigen Phase in einem einzigen Schritt durch Mischen unter Scherbedingungen;

wodurch die einkettigen, phospholipidfreien, paucilamellaren Lipidvesikel mit lokalisierter Ladung, die in ihren Zentralhöhlen einen mit Wasser nicht mischbaren, öligen Stoff aufweisen, ohne die Ausbildung einer abtrennbaren, lamellaren Phase gebildet werden.

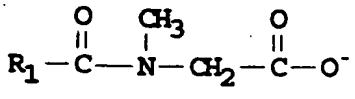
10. Verfahren gemäß Anspruch 8 oder Anspruch 9, worin das Betain ein Betain folgender Struktur enthält oder daraus besteht:



worin R_2 ein langkettiger Fettsäureester, z. B. Propyloleat, mit folgender Struktur ist:



11. Verfahren gemäß Anspruch 8 oder Anspruch 9, worin das Sarkosinamid ein Sarkosinamid folgender Struktur enthält oder daraus besteht:



12. Verfahren gemaß Anspruch 8 oder Anspruch 9, worin die lipophile Phase weiterhin ein Sterol, z. B. Cholesterol, enthält oder daraus besteht.

13. Verfahren gemäß Anspruch 8 oder Anspruch 9, worin die lipophile Phase weiterhin einen ladungerzeugenden, amphiphilen Stoff enthält oder daraus besteht.

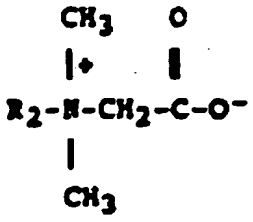
Revendications

1. Vésicules lipidiques paucilamellaires constituées de 2 à 10 bicouches de lipides sous la forme d'enveloppes sphériques séparées par des couches aqueuses, lesdites bicouches de lipides comprenant un agent tensioactif non phospholipidique à chaîné unique et porteur de charges localisées choisi dans le groupe constitué par des bétaines et des sarcosinamides anioniques comme lipide primaire, et des matériaux quellconques solubles dans les lipides et devant être incorporés dans les bicouches de ces vésicules lipidiques

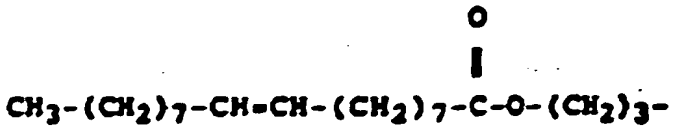
2. Les vésicules lipidiques paucilamellaires selon la revendication 1, dans lesquelles les bicouches lipidiques entourent une cavité centrale amorphe remplie de milieu aqueux.

3. Les vésicules lipidiques paucilamellaires selon la revendication 1, dans lesquelles une cavité centrale amorphe remplie de milieu huileux.

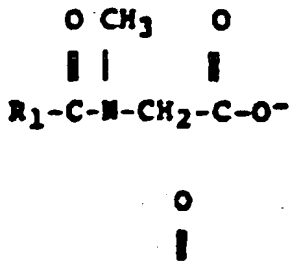
4. Les vésicules lipidiques paucilamellaires selon l'une quelconque des revendications 1 à 3 dans lesquelles ladite bétaine comprend une bétaine présentant la structure :



dans laquelle R_2 est un ester d'acide gras à longue chaîne, par exemple l'oléate de propyle présentant la structure :



5. Les vésicules lipidiques paucilamellaires selon l'une quelconque des revendications 1 à 3 dans lesquelles ledit sarcosinamide anionique comprend un sarcosinamide présentant la structure :



dans laquelle R_1-C représente le groupe carbonyle d'un acide gras à longue chaîne renfermant de 12 à 20 atomes de carbone, ledit acide gras à longue chaîne étant par exemple choisi parmi : l'acide laurique. les acides gras renfermant de 14 à 20 atomes de carbone et leurs mélanges.

6. Les vésicules lipidiques paucilamellaires selon l'une quelconque des revendications 1 à 3 dans lesquelles ladite phase lipophile comprend en outre un stérol par exemple le cholestérol.

7. Les vésicules lipidiques paucilamellaires selon l'une quelconque des revendications 1 à 3 dans lesquelles ladite phase lipophile comprend en outre un corps lipophile-hydrophile producteur de charge.

8. Un procédé de préparation des vesicules lipidiques paucilamellaires selon la revendication 2 comprenant essentiellement les étapes suivantes

A. Formation d'une phase lipophile non aqueuse exempte de solvant comprenant un agent tensioactif non phospholipidique à simple chaîne et porteur de charges localisées choisi dans le groupe constitué par des bétaines et par des sarcosinamides anioniques et des matériaux solubles dans les lipides et devant être incorporés dans lesdites vésicules lipidiques,

B. Formation d'une phase aqueuse constituée d'un solvant aqueux et de matières quelconques solubles en milieu aqueux et devant être encapsulées; et

C. Réunion de ladite phase lipophile non aqueuse avec un excès de ladite phase aqueuse en une seule étape dans des conditions de mélange avec cisaillement ;

lesdites vésicules lipidiques paucilamellaires se formant en évitant la formation d'une phase lamellaire séparable.

9. Un procédé de préparation de vésicules lipidiques paucilamellaires rempli de milieu aqueux selon la revendication 3 renfermant une matière huileuse non miscible à l'eau dans leurs cavités centrales amorphes comprenant essentiellement les étapes suivantes :

A. Formation d'une phase lipidique non aqueuse exempte de solvant comprenant un agent tensioactif non phospholipidique à simple chaîne et porteur de charges localisées choisi dans le groupe constitué par des bétaines et par des sarcosinamides anioniques et des matériaux solubles dans les lipides et devant être incorporés dans lesdites vésicules lipidiques ;

B. Formation d'une phase aqueuse constituée d'un solvant aqueux et de matières quelconques solubles en milieu aqueux et devant être encapsulées; et

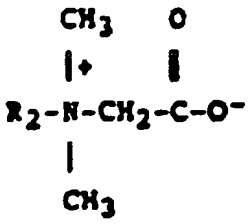
C. Formation d'une phase huileuse d'une matière huileuse non miscible à l'eau et de toute matière soluble dans celle-ci et devant être incorporée dans ladite vésicule lipidique ;

D. Formation d'une phase lipophile par mélange de ladite phase lipidique et de ladite phase huileuse ;

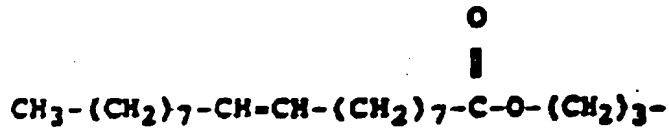
E. Réunion de ladite phase lipophile avec un excès de ladite phase aqueuse en une seule étape dans des conditions de melange avec cisaillement ;

lesdites vésicules lipidiques paucilamellaires non phospholipidiques à chaîne unique et porteurs de charges localisées renfermant une matière huileuse non miscible à l'eau dans leurs cavités centrales étant formées en évitant la formation d'une phase lamellaire séparable.

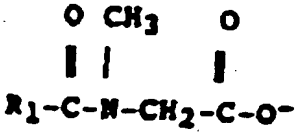
10. Le procédé selon la revendication 8 ou 9 dans lequel ladite bétaine comprend une bétaine présentant la structure :



dans laquelle R_2 est un ester d'acide gras à longue chaîné, par exemple l'oléate de propyle présentant la structure :



11. Le procédé selon la revendication 8 ou 9 dans lequel ledit sarcosinamide comprend un sarcosinamide présentant la structure :



12. Le procédé selon la revendication 8 ou 9 dans lequel ladite phase lipophile comprend en outre un stérol, par exemple le cholestérol.

13. Le procédé selon la revendication 8 ou 9 dans lequel ladite phase lipophile comprend en outre un dérivé lipophile-hydrophile producteur de charge.